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THE GENESIS OF THE ORGANIZATION OF THE INSECT EGG. II

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5. Interaction of Nucleoplasm and Cytoplasm

There are phenomena that occur during the growth period that suggest how masses of cytoplasm that are differentiated both morphologically and physiologically may arise in the cortical layer of the insect egg. It has been suggested that "most of the differentiations of the egg cytoplasm have arisen during the ovarian history of the egg and as a result of the interaction of nucleus and cytoplasm; . . .,"²⁷ and with this we fully agree, but our problem is to determine the nature of this interaction and in what ways it may take place.

During every mitosis there is a more or less thorough mixing that involves the chromatin as well as other nuclear constituents, since chromatin-diminution is a normal histological process. Interchanges between nucleus and cytoplasm, therefore, occur during the two multiplication periods that precede the formation of oocytes. Abundant opportunity is thus offered for factors in the chromosomes to exert an influence upon the cell as a whole. A similar and probably even greater discharge of chromatic

²⁷ Conklin, 1916, "Heredity and Environment," New York.

and other nuclear substances into the cytoplasm occurs during the maturation divisions of the egg, but this period may be neglected in this connection, since the organization with which we are concerned is already established before maturation takes place. Even when the nuclear membrane is intact, substances undoubtedly pass in and out of the nucleus much as they do through the cell membrane, and as in the latter, the nuclear membrane may change in permeability at different times, these changes being due to chemical processes taking place within the nucleus or in the cytoplasm. Such changes occur more often during periods of cell activity than at other times and thus we should expect pronounced interaction throughout the growth period of the oocytes.

Besides gradual, and for the most part invisible, interchanges of this sort there may be actual transference of visible masses of chromatin from the nucleus to the cytoplasm. These chromatin granules that escape into the cytoplasm have been called "chromidia" and are supposed to play a part in cytoplasmic differentiation.

A peculiar process of interchange by means of secondary nuclei is exhibited by certain insects, especially Hymenoptera.²⁸ This process has been studied most carefully in the carpenter ant, *Componotus herculeanus* var. *pennsylvanica* (Fig. 11). At an early stage in the growth of the oocyte small vesicles containing a few granules of chromatin appear near the oocyte nuclei. These "secondary nuclei" appear to arise as buds from the primary nucleus, but no one has yet actually observed their formation in this way. It has also been suggested that they may be epithelial cells that have invaded the oocyte, but this seems very improbable. The writer has reached the conclusion that they consist of nuclear materials that have been given off into the cytoplasm and have there become enclosed by membranes which give them a nuclear-like appearance. As the oocyte increases

²⁸ Blockmann, 1886, *Festsch. nat.-med. Verein zu Heidelberg*; Buchner, 1913, *Biol. Centrbl.*, Bd. 33; Hegner, 1915, *Journ. Morph.*, Vol. 26.

in size the secondary nuclei increase in number until they entirely surround the primary nucleus, forming several layers. When the oocyte has nearly reached its full growth they begin to migrate from the group near the anterior end of the oocyte and become scattered throughout

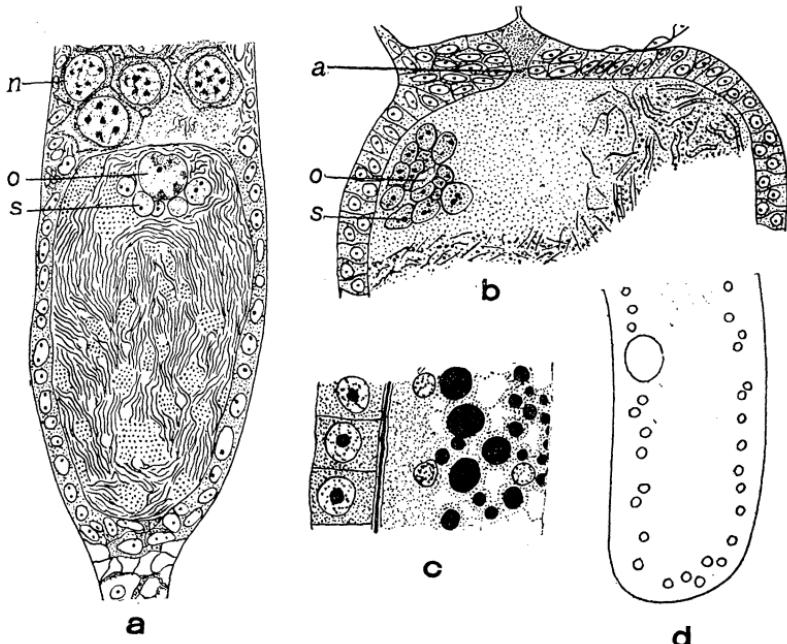


FIG. 11. Secondary nuclei in the oocytes of the carpenter ant (a, b, c) and the Hymenopterous gall-fly, *Rhodites ignota* (d). (Hegner, 1915.)

a. Oocyte (o) shortly after secondary nuclei (s) begin to appear. n = nurse cells.

b. Older oocyte showing oocyte nucleus (o) surrounded by secondary nuclei (s). a = connection between oocyte and nurse chamber.

c. Part of a still older oocyte showing follicular epithelium, yolk globules (black), and secondary nuclei.

d. Part of an oocyte of *Rhodites* showing primary nucleus (large circle) and secondary nuclei (small circles).

out the egg, forming a rather regular layer a short distance beneath the periphery. The further history of these bodies is not certain, but they undergo changes by which they lose their identity, since they can not be found in fully grown eggs. Their function is likewise problematical. They may take part in the formation of germ-line determinants which probably occur in the eggs of

this ant;²⁹ they may aid in changing the substances furnished by the nurse cells into material available for the embryo;³⁰ or they may have something to do with the formation of yolk.³¹ It is also possible that they may control differentiation in the peripheral layer of cytoplasm and thus provide a method of nuclear control of the organization of the egg. The last hypothesis may be objected to on the grounds that the secondary nuclei appear to be irregularly distributed and that they are known to occur in only a few species of insects.

Another possible way in which the initial organization of the insect egg may arise is through the activities of mitochondria. The rather constant presence of these bodies in the cytoplasm of almost all types of cells indicates that they may be of considerable importance in the process of differentiation. If they take part in the genesis of egg organization they then may play the rôle attributed to them by certain investigators of being the cytoplasmic bearers of hereditary factors corresponding in this respect to the nuclear bodies of similar function, the chromosomes.

The most striking differentiation in the cytoplasm of the insect egg is that which involves the germ-line determinants. As stated above, we do not know for certain in any case the origin of the peculiar cytoplasmic mass that contains these determinants, but a number of hypotheses have been suggested. In *Miastor*, for example, the following is offered to account for the appearance of the "pole-plasm" in the fully developed oocyte.³²

It may be distinguished from the rest of the egg contents by its position at the posterior end and because of its affinity for certain dyes. It appears shortly before the maturation division is initiated, but no transition stages have been discovered—it has been either present or entirely absent in the preparations thus far studied. If we consider the history of this substance from the formation of the primordial germ

²⁹ Hegner, 1914, *Journ. Morph.*, Vol. 26.

³⁰ Marshall, 1907, *Zeit. wiss. Zool.*, Bd. 86.

³¹ Loyez, 1908, *C. R. Assoc. Anat.*, 10 Reunion, Marseille.

³² Hegner, 1914, "Germ-Cell Cycle in Animals," New York.

cell to the growth period of the oocytes produced by this primordial germ cell, we may conclude that at the time the multiplication period ends the pole-plasm has become equally distributed among the sixty-four oogonia. Then ensues the growth period during which the pole-plasm can not be distinguished. Later, however, just before maturation, pole-plasm substance reappears which is equal in amount to that contained in the primordial germ cell of the preceding generation or to that contained in all of the sixty-four oogonia which descended from that primordial germ cell. That is, the pole-plasm of the oocyte under discussion has in some way increased until its mass is sixty-four times as great as that of the oogonium before the growth period began. How this increase has taken place can only be conjectured. The pole-plasm in the oogonium may have produced new material of its own kind either by the division of its constituent particles or by the influence of its presence.

The influence of a specialized mass of cytoplasm upon the chromatin is very well illustrated by the inhibition of chromatin-diminution in *Miastor* and *Ascaris*. In *Miastor* nuclear division is normal until at the four-cell stage one nucleus reaches the pole-plasm at the posterior end (Fig. 12, *a*, IV.). During the succeeding mitosis this nucleus, which is apparently under the control of the pole-plasm, does not undergo chromatin-diminution, whereas the other three do. One of the daughter nuclei resulting from the division of this undiminished nucleus remains entirely within the pole-plasm and is cut off from the rest of the egg with this specialized mass of cytoplasm as the primordial germ cell (Fig. 12, *b*). This nucleus always retains the full amount of chromatin; but its sister nucleus, which remains in the egg and is thus separated from the direct influence of the pole-plasm, undergoes diminution at the next mitosis.

A similar segregation of specialized cytoplasm in the primordial germ cells occurs also in certain other insects and in copepods, but no diminution process has yet been discovered in them. In *Ascaris*, where chromatin-diminution was first reported,³³ there is evidently a segregation of germinal cytoplasm at each cleavage division up to the sixteen-cell stage, when it is all confined in one cell, the

³³ Boveri, 1887, *Anat. Anz.*, Bd. 2.

primordial germ cell. This cytoplasm, which is not visibly different from the rest, as in *Miastor*, appears to inhibit diminution in every nucleus that comes within its

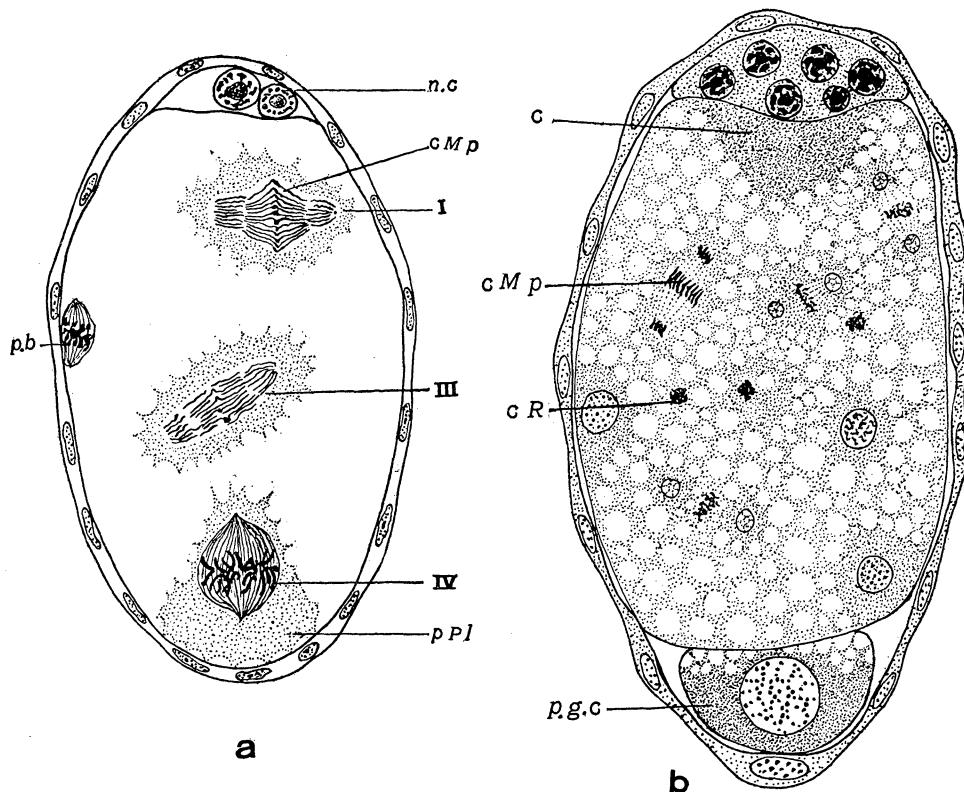


FIG. 12. a. Longitudinal section through an egg of *Miastor* showing chromatin diminution in nuclei I. and III. but not in nucleus IV. which has come under the influence of the pole-plasm (*p Pl*). Nucleus II. does not appear in this section. *cMp* = chromatin that is cast off into cytoplasm. *nc* = nurse cells. *pb* = polar body. (Kahle, 1908.)

b. Longitudinal section through an egg of *Miastor*, showing primordial germ cell (*p.g.c.*), nuclei undergoing chromatin diminution (*cMp*), and the remains of chromatin cast out into the cytoplasm (*cR*). *c* = cytoplasm elaborated by nurse cells above. (Hegner, 1914.)

immediate influence as indicated by experimental studies on dispermic and centrifuged eggs.³⁴ In this respect it resembles the pole-plasm of *Miastor*.

³⁴ Boveri, 1910, *Arch. Ent.-mech.*, Bd. 30; Boveri, 1910, *Festschr. R. Hertwig.*, Bd. 3.

6. Mendelian Factors and Cytoplasmic Organization

The central biological problem of the present time is the method of evolution, and a knowledge of the mechanism of heredity has long been recognized as necessary for its solution. The results derived from breeding experiments with the fruit fly, *Drosophila ampelophila*, have dominated the field of genetics for the past five years, but although of very great interest and importance, their evolutionary significance is not yet certain. To be of primary value from this viewpoint it is necessary to prove that new species may arise by means of Mendelian characters (mutations) such as white eye, miniature wing, club wing, etc. Since no one has ever been able to define satisfactorily what a species really is and hence what characters should be considered of specific value, this is a difficult problem.

The definitions given by two of our foremost authorities, one a systematist and the other a geneticist, are as follows: The systematist writes:³⁵

Forms of animals which present distinct assemblages of characters, in form, color and arrangement of parts under natural conditions, which are recognizable from descriptions and figures, should receive distinctive names and be catalogued, provided, of course, that the assemblage of characters includes all ontogenetic changes. If, in the examination of abundant material from different natural environments, we find these characters fairly constant, the forms may properly be called species; if not, varieties or races.

The geneticist writes:³⁶

Species may thus be distinguished by peculiarities of form, of number, of geometrical arrangement, of chemical constitution and properties, of sexual differentiation, of development and of many other properties. In any one or in several of these features together, species may be found distinguished from other species.

The mutations that have appeared in *Drosophila* do not become recognizable until a late stage in the life history of the individual, and are about the last characters

³⁵ Williston, 1908, AMER. NAT., Vol. 42.

³⁶ Bateson, 1913, "Problems of Genetics."

to appear in the individual development. They for the most part affect the size and shape of the wings, the size, shape and color of the eyes, and the color of the body.

If a systematist were asked whether these new races of *Drosophila* are comparable to wild species, he would not hesitate for a moment. He would call them all one species. If he were asked why, he would say, I think, "These races differ only in one or two striking points, while in a hundred other respects they are identical even to the minutest details." He would add, that as large a group of wild species of flies would show on the whole the reverse relations, viz., they would differ in nearly every detail and be identical in only a few points.³⁷

This point of view seems justified, since the foremost dipterologist in this country, a man who has named over one thousand species and genera, mostly of flies, says regarding the results of certain experiments carried on with *Drosophila* by one of his colleagues.³⁸

But I think it is absolutely certain—and I speak as an entomologist fairly familiar with flies—that it would be impossible to produce species of his sports even though they were bred for a thousand years.³⁹

In talking over this species question with one who has had considerable experience in systematic work⁴⁰ it became clear that although as a rule only a few of the more conspicuously contrasting characters are selected for descriptive purposes, as a matter of fact the individuals of different species are often different in practically every morphological characteristic. One who is very familiar with these species will realize these differences at once, although many of them are of such a nature that they can not be described so that any one else will recognize them. There seems to be no difficulty, however, in finding numerous describable contrasting characters in *Drosophila*, since at least fifty-nine are included in the descriptions of two recently named species⁴¹ that were selected

³⁷ Morgan, 1916, "Critique of the Theory of Evolution."

³⁸ Dr. F. E. Lutz.

³⁹ Williston, 1908, AMER. NAT., Vol. 42.

⁴⁰ Dr. Alexander G. Ruthven.

⁴¹ Sturtevant, 1916, *Annals Ent. Soc. Amer.*, Vol. 9.

at random, *D. superba* and *D. projectans*, and these characters relate to almost every part of the body. Many other differences would probably also be found between the physiological processes and general activities of the adults and between the morphological and physiological characteristics of the embryos, larvæ and pupæ of the two species if they were compared from these standpoints. It has been shown that the factor for the character club wing affects not only the character that gives this mutation its name, but also other characters, for example the presence or absence of a pair of spines on the sides of the thorax, these being always absent when the factor for club wing is present.⁴² It is possible that the combination of a number of such factors as that for club wing would ultimately satisfy the requirements of systematic entomologists and that new species could then be made up in the laboratory. Such mutations might therefore be of evolutionary value. If, however, these mutations fail to furnish characters of specific rank, or characters that may lead to the formation of new species, we must conclude that they are not of evolutionary significance, and look elsewhere for the factors that are responsible for specific characters and that may undergo changes which lead to transmutation.

Factors of this sort may lie in the chromosomes or in the cytoplasm, but they are probably the results of interaction between chromosomes and cytoplasm. As pointed out above, interaction of this sort has abundant opportunity to operate during the germ-cell cycle. The cytoplasmic differentiations resulting from the metabolic processes that culminate in the formation of an egg ready to undergo maturation are very striking in the case of insects, as indicated by observations and experiments on the eggs of chrysomelid beetles, and there seems to be no valid reason why the eggs of these beetles are different in their type and complexity of organization, both morphological and physiological, from those of *Drosophila*; for while we do

⁴² Morgan, Sturtevant, Muller, and Bridges, 1915, "Mechanism of Mendelian Heredity."

not know much about the growth of the egg and embryological development of this genus of flies, we do know that these processes in certain other flies resemble those of beetles.

If the Mendelian factors are located in the chromosomes, it is evident that they may exert an influence upon the entire contents of the egg, (1) during the mitotic divisions of the oogonia, (2) during the so-called resting stages of the oogonia, and (3) during the growth of the oocytes. It is also clear that all of the factors carried by the chromosomes have an equal opportunity to interact with the cytoplasm and not alone those that remain within the egg after the elimination of chromosomes during maturation. The adult, however, that develops from the egg, whether fertilized or unfertilized, exhibits only those detailed characteristics whose genetic factors are supposed to be located in the chromosomes remaining in the egg after maturation, or in those that are brought in by the sperm. This seems to indicate that none of these factors has any permanent influence upon the egg organization during the growth of the oocyte and until maturation is completed.

It seems impossible to ignore the chromosomes or even to locate the principal factors of heredity in any other cell bodies. It may therefore be necessary to reconstruct our ideas of chromosome architecture and thereby abandon the theory that these bodies consist of a linear series of factorial determiners for certain ferments and of nothing else. It may be possible to separate our hypothetical factors into two groups, (1) those responsible for such characteristics as the polarity, bilaterality and "pattern" of the egg, and (2) those that control mutations that appear at a late period in the life history like those that are so abundant in *Drosophila*. Perhaps the latter may be anchored to the chromosomes as has recently been suggested;⁴³ the main portion of the chromosomes might then represent the foundation for the factors responsible for the organization of the egg and the attached masses of

⁴³ Goldschmidt, 1917, *Genetics*, Vol. 2.

ferments might constitute the factors responsible for the modification of embryonic, larval and adult characters—factors such as have been employed for experimental breeding purposes by most geneticists. According to this hypothesis it would probably be necessary to consider the main portions of each chromosome as sufficient for the production of an entire organism. The fact that the group of factors carried by any one chromosome in *Drosophila* controls characters that are not restricted to any definite part of the body gives weight to this assumption.

Most geneticists are accustomed to deal with adult characters only, and on this account pay very little or no attention to the eggs, embryos and larvae of the species they are experimenting with. But the eggs, embryos and larvae contain all the factors for these adult characters, both those that are realized and those that are inhibited either by internal or external causes, and they may exhibit characters that make it possible to separate different lines although the adults may be indistinguishable. Furthermore, taxonomists have long recognized the value of embryonic characters as an aid in determining species.

We should always be careful to distinguish between the parts of the egg that are of hereditary significance and those that are not. Thus the shell or chorion of the silk-worm egg has been discussed under the heading of "cytoplasmic inheritance," whereas it is not a vital part of the egg, but, being secreted by the epithelium of the ovarian tube, is a well-defined characteristic of the adult female and its coloration, which follows the laws of Mendelian inheritance,⁴⁴ is controlled by maternal factors.

Such fundamental characteristics as polarity, symmetry, and pattern, which are so clearly exhibited by the eggs of insects and certain other animals, are much more difficult to study than adult characters and are probably not so easily modified. If any or all of them are carried over from one generation to another in the cytoplasm we have then a real instance of cytoplasmic inheritance. Even if this is the case the chromosomes doubtless exert

⁴⁴ Toyama, 1913, *Journ. of Genetics*, Vol. 2.

an influence upon the cytoplasm during the oogonial and growth periods of the egg, and a study of the genesis of cytoplasmic organization may lead to data that will help us solve this difficult problem.

If the polarity of the oocyte when recognizable is not inherited, *i. e.*, if it is not transmitted to the primordial germ cells by the egg, and retained by the oogonia, it must arise *de novo* just before or during the growth period. One observer⁴⁵ has found that in certain beetles the position of the spindle remains, resulting from the differential divisions that precede the formation of the oocyte, indicates the polarity of the ultimate organism, but he does not tell us how this "polarité predifferentielle" is brought about. In all insects the end of the egg directed toward the head of the mother becomes the anterior end of the offspring. This is also the pole of the egg lying next to the nurse cells or that is closest to the nurse-cell chamber. This relation between oocyte and nurse-cells may be the determining factor in the polarity of these eggs and, if so, would indicate that polarity here is due to environment. How this relation could influence the polarity may be explained by means of axial gradients of metabolism, such gradients in this case being produced by greater external stimulation at the end near the nurse-cell chamber where nutritive substances are elaborated and added to the oocyte. By this theory of metabolic gradients, differentiation along an antero-posterior axis can be accounted for and further differentiations of a morphological and physiological nature would result from "chemical transportative correlation between the different parts."⁴⁶

We should not lose sight of the fact, however, that these hypothesized physiological activities require protoplasm as a material basis and that their results depend upon the character of this protoplasm. If polarity is established at the stage suggested above, it follows a long series of nucleo-cytoplasmic interactions which have no doubt resulted in the differentiation and localization of numerous

⁴⁵ Govaerts, 1913, *Arch. Biol.*, Tome 28.

⁴⁶ Child, 1916, *Science*, Vol. 43.

kinds of cytoplasm. The appearance of a definite polarity might lead in some way to diffusion processes and the circulation of secretions resulting in further specializations and localizations. One stage seems to initiate the next stage in the series of processes that accompany the visible changes in the growth and development of the egg, and the character of these processes is of course due to the specificity of the protoplasm.

That the cytoplasm may exert a controlling influence upon the chromatin has been demonstrated in several instances. For example, we know that the chromatin-diminution processes during the early cleavage of both *Ascaris* and *Miastor* are controlled by the cytoplasm and that in these animals the germ-cell nuclei retain the full amount of chromatin because of the germ-cell cytoplasm they chance to encounter.

Probably the peculiar distribution of the chromosomes at certain stages in the life histories of certain aphids, phyloxerans, and Hymenoptera is also controlled by the cytoplasm. In the aphid, *Aphis saliceti*, the first maturation division is visibly differential both as regards the chromosomes and the cytoplasm.⁴⁷ The mitochondria congregate at one end of the dividing spermatocyte; this process is accompanied by a greater accumulation of cytoplasm at this end so that cell division results in one large cell containing all of the mitochondria and about two thirds of the cytoplasm, and one functionless small cell. The large cell also receives three chromosomes; the small cell only two.

The peculiar maturation divisions in the males of the honeybee⁴⁸ and hornet,⁴⁹ during which one ultimate spermatogonium gives rise to only one spermatozoon instead of the usual four, may also be the result of cytoplasmic control. The cytoplasm may likewise be responsible for the passing of a sex chromosome into the polar body during the maturation of the egg of certain aphids at the end of the summer season.⁵⁰ Such eggs must be fertilized

⁴⁷ Baehr, v, 1909, *Arch. für Zellf.*, Bd. 3.

⁴⁸ Meves, 1907, *Arch. mikr. Anat.*, Bd. 70.

⁴⁹ Meves and Duesberg, 1908, *Arch. mikr. Anat.*, Bd. 71.

⁵⁰ Morgan, 1909, *Journ. Exp. Zool.*, Vol. 7.

before they will develop, and always produce males. Many other peculiarities in the behavior of chromosomes that have been reported from time to time may also be due to the influence of the environment (cytoplasm), and there seems to be no reason why factors carried by the chromosomes should not be affected by the cytoplasm as well as are entire chromosomes.

By the interaction of Mendelian factors with the cytoplasm during the germ-cell cycle, it is even possible to explain the fact that "crossing over" occurs in the females of *Drosophila*, but not in the males.⁵¹ In the latter, the spermatocytes do not pass through a pronounced growth period, and hence there is comparatively little nucleo-cytoplasmic interaction, and since the cytoplasm carried by the sperm may be considered negligible, the factors borne by its chromosomes are not interfered with. In the female, however, there is ample opportunity for such interaction during the growth period, and factors at this time may be influenced by the cytoplasm or may influence the cytoplasm in such a way as to cause an irregular distribution of chromosomal factors.

To the writer the following conclusions seem justified. The insect egg at the time of maturation is a mosaic of differentiated cytoplasmic areas predetermined to develop into definite parts of the embryo. This organization has resulted from the interaction of nucleus and cytoplasm during the germ-cell cycle. Such interaction is taking place at all times, but is visible only when such processes as the protrusion of chromidia or chromatin-diminution occur. The many cases of cytoplasmic control over chromatin behavior, and the apparent failure of the factors for the characters commonly used by geneticists to influence the egg organization, indicate the importance of more careful studies of the genesis of this organization. The importance of such studies is emphasized by the possibility that they may help toward the solution of the problem of the method of evolution.

⁵¹ Morgan, Sturtevant, Muller, and Bridges, 1915, "Mechanism of Mendelian Heredity."